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EXPERIMENTAL MYCOTOXIC NEPHROPATHIES AND BALKAN ENDEMIC NEPHROPATHY

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Nearly half a century after its first recognition in Bulgaria, the cause of Balkan endemic nephropathy (BEN) and its associated higher incidence of urinary tract tumours remains obscure. Even 20 years ago some current ideas concerning viruses, bacterial pathogens, heavy metals from geological sources, and nephrotoxic medicinal plants such as *Aristolochia*, were discounted in a comprehensive review (1) as not fitting the mosaic of disease incidence. However, as part of the present new review of BEN it may be helpful to reflect on the topic of nephrotoxic mycotoxins, which has long been a popular source of putative aetiological agents. The present author does so, following 20 years of active research interest both in BEN 'in the field' in Croatia and Bulgaria, and in experimental science of some nephrotoxic mycotoxins of possible relevance.

Ochratoxin A has been the main focus of international research concerning BEN because of its role as a cause of the Danish porcine nephropathy that became of economic concern in the bacon industry in the mid 20th century. In that case, the fungus (now known as *Penicillium verrucosum*, naturally producing the toxin in northern European latitudes) was clearly demonstrated as a common spoilage microorganism of poorly-stored barley. Its occurrence was correlated with the presence of its key metabolite ochratoxin A and experiments in pigs with the pure toxin and experimentally spoiled grain confirmed that ochratoxin A was clinically toxic at concentrations commonly occurring in years of poor harvest weather. Thus a satisfactory principal cause and effect correlation was proved for ochratoxin A concerning that porcine nephropathy.

It became reasonable, therefore to suggest extrapolation from Danish mycotoxic porcine nephropathy to the mysterious and idiopathic BEN in the early 1970s (2). However, it may have slanted some investigations of the human disease away from a search for its cause and towards an attempt to show that ochratoxin A also fits as a cause of BEN. Further, the claim at that time of similar renal histopathology between porcine nephropathy and BEN has since received rather little direct challenge. Of course there are features in common. But it is usually ignored that nephropathy in growing pigs is a renal hypertrophy, often quite marked and correlated with impaired live weight gain, while BEN is a very silent renal atrophy with accompanying fibrosis in peasant farmers who do not seem to have a history of chronic poor health. Interestingly, there does not seem to be nephropathy in the pigs that the BEN subjects almost all keep and which are likely to eat a diet of inferior quality.

The situation is the more complicated by the frequent co-occurrence of urinary tract tumours with the renal atrophy of BEN, and the clear role of ochratoxin A in causing renal tumours in rats. Ochratoxin A thus seems to have an array of toxic attributes ideally suited to connection with many aspects of the Balkan disease syndrome. Therefore it has been important to study the toxicology of ochratoxin A and other nephrotoxic metabolites of food/feed-spoilage moulds in experimental animals with respect to both renal tubular morbidity and tumourigenesis.

Of course, ochratoxin A is a very toxic substance. Perhaps it is too toxic and broady toxic to cause the specific and silent BEN. Direct renal insults as tubule epithelial cell loss appear to be mainly necrotic. Claims of evidence for loss by apoptosis *in vivo* seem weak since they are not based on specific staining. Indeed, apoptosis is not readily compatible with carcinogenicity. Further, our recent studies have shown that administration to rats by oral gavage causes much greater damage than when ingested *via* feed (3). Some experimentation may therefore have given exaggerated effects. Similarly, a recent 1-year experiment in pigs fed continuously on a diet containing 800ppb ochratoxin A revealed a slight effect on growth and a rather mild renal histopathology. It was judged that the animals would not have been recognised by routine meat inspection at slaughter as subjects with ochratoxicosis (4). In contrast a similar dose given to pigs over a shorter time, but augmented with the common mycotoxin penicillic acid in the diet, showed much more severe effects (5). Experimentation that can be meaningful in terms of risk assessment for humans and applicability to the BEN/ urinary tract tumour complex should therefore include diets which have a heterogeneous mycotoxin composition relevant to the range of natural circumstances. It is also questioned whether experimentation in rats dosed with

ochratoxin A on an increasing body-weight basis during life also exaggerates response relative to normal dietary intake and body conformation in humans, especially concerning effects on excretory organs.

Another experimental mycotoxic nephropathy is that caused in rats by crude water extract of the food-spoilage mould, now known as *Penicillium polonicum,* which is common in the Balkans. This causes extensive apoptosis in proximal tubule epithelia (6). It is also potently mitogenic, resulting in huge and persistent karyomegaly (7). The clear demonstration of tissue-specific rat renal tubule apoptosis by a common mould stimulated the idea that silent apoptosis is an attractive mechanism by which BEN might arise and progress. *P. polonicum* nephrotoxin might only be a model for a similar effect from an otherwise unexpected source (6). Very early stages in BEN are difficult to specify. The author is not aware of any confident description of such; so-called early stages in a kidney with atrophy may actually be early end-stage in the whole 'lifetime' aetiology of BEN. Strangely, the same mould has been shown to alter the debrisoquine-metabolising capability in rats (8), so as to give a scientific base for questioning the fidelity of a statistical finding of nephropathy patients tending also to be faster metabolisers of debrisoquine.

Current focus in the author's laboratory is collaborative research with others in Western Europe towards fundamental understanding of aspects of ochratoxin toxicity. This involves broad study of rat gene expression changes using 1000 gene DNA microarrays. We would be pleased to include analogous study of small amounts of suitable ultra-fresh renal tissue from BEN subjects that may become available. This might help in comparing the human disease with some known effects of ochratoxin A to see the extent of fit. It could also provide a valuable knowledge base concerning the gene expression status of human tissue captured during the development of BEN.

Always it is necessary to remember that the incidence of BEN is highly mosaic both within and between endemic villages. Any putative cause must fit this, as also the strong familial character of the disease. Also, aetiological study should strive for rigorous proof analogous to that prescribed in the late 19th century by Robert Koch for establishing the cause of infectious disease. In the Balkans ochratoxin A has been found in cereal commodities in small amounts from time to time, much as it has in some other regions of the world, and sometimes much emphasis is placed on the occasional higher than usual concentration. However, unlike in northern European latitudes, *P. verrucosum* seems not to be a toxigenic mould. *Aspergillus ochraceus*, the source of the original discovery of ochratoxin A in South Africa in the early 1960s, is not common and rather few isolates have been found to produce significant amounts of the toxin. Some other ochratoxigenic *Penicillium* spp. have been found, but generally the source of ochratoxin A in human food in the Balkans is obscure (9). This illustrates the neglect of rigorous forensic study of BEN for far too long and does not compel confidence in convicting nephrotoxic mycotoxins of a causative role in BEN beyond reasonable doubt. However, even if, for whatever reason, the incidence of BEN can be shown, fortunately, to be in decline it is still important to find the cause as a model for addressing other apparently intractable aetiological questions in human medicine.

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